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A Genome-wide Association Study of Bipolar Disorder with Comorbid Eating Disorder Replicates the SOX2-OT Region

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Abstract

Background—Bipolar disorder is a heterogeneous mood disorder associated with several important clinical comorbidities, such as eating disorders. This clinical heterogeneity complicates the identification of genetic variants contributing to bipolar susceptibility. Here we investigate comorbidity of eating disorders as a subphenotype of bipolar disorder to identify genetic variation that is common and unique to both disorders.

Methods—We performed a genome-wide association analysis contrasting 184 bipolar subjects with eating disorder comorbidity against both 1,370 controls and 2,006 subjects with bipolar disorder only from the Bipolar Genome Study (BiGS).

Results—The most significant genome-wide finding was observed bipolar with comorbid eating disorder vs. controls within SOX2-OT ($p=8.9\times10^{-8}$ for rs4854912) with a secondary peak in the adjacent FXR1 gene ($p=1.2\times10^{-6}$ for rs1805576) on chromosome 3q26.33. This region was also the most prominent finding in the case-only analysis ($p=3.5\times10^{-7}$ and 4.3×10^{-6}, respectively).

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Several regions of interest containing genes involved in neurodevelopment and neuroprotection processes were also identified.

Limitations—While our primary finding did not quite reach genome-wide significance, likely due to the relatively limited sample size, these results can be viewed as a replication of a recent study of eating disorders in a large cohort.

Conclusions—These findings replicate the prior association of SOX2-OT with eating disorders and broadly support the involvement of neurodevelopmental/neuroprotective mechanisms in the pathophysiology of both disorders. They further suggest that different clinical manifestations of bipolar disorder may reflect differential genetic contributions and argue for the utility of clinical subphenotypes in identifying additional molecular pathways leading to illness.

Keywords

eating disorders; bipolar disorder; genome-wide association (GWAS); comorbidity; SOX2-OT

INTRODUCTION

Bipolar disorder is a severe mood disorder with an estimated heritability of 60–93% (Kieseppa et al., 2004; Lichtenstein et al., 2009; McGuffin et al., 2003; Taylor et al., 2002). Genome-wide association (GWA) studies of large samples have recently identified several strong candidates for susceptibility genes, including ADCY2, ANK3, CACNA1C, NCAN, ODZ4, and TRANK1 (Cichon et al., 2011; Ferreira et al., 2008; Green et al., 2013; Chen et al., 2013; Muhleisen et al., 2014; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011), although the pathways by which genetic variants impact risk are complex and remain largely unknown. Bipolar disorder also presents with complex, highly variable clinical manifestations, including several important comorbidities that constitute a wide range of disorder subtypes (MacQueen et al., 2005). This phenotypic heterogeneity impedes the clarification of genetic variants contributing to susceptibility, since a given sampling of bipolar patients likely consists of multiple different subtypes, each with a unique genetic architecture (Alda, 2004; Alda et al., 2009). The use of subphenotypes derived from clinical factors known to be associated with the disorder may establish more homogeneous subgroups of patients with distinct underlying genetic risk factors (Saunders et al., 2008). While several potentially important subphenotypes of bipolar disorder have been identified as part of the characteristic symptomatology or comorbidity (MacQueen et al., 2005; Saunders et al., 2008), few GWA analyses have utilized clinical subphenotypes for bipolar disorder (Greenwood and Kelsoe, 2013; Swaminathan et al., 2015; Winham et al., 2014).

Mounting evidence suggests a strong connection between the etiology of bipolar disorder and that of eating disorders. Patients with bipolar disorder have elevated rates of eating disorders (McElroy et al., 2013; McElroy et al., 2006; McElroy et al., 2005), with eating disorder comorbidity being more commonly observed among female than male bipolar patients (Kawa et al., 2005; McElroy et al., 2011), consistent with observations in the general population (Hudson et al., 2007). While rates of binge eating behaviors range from 13% to 38% in bipolar disorder (Kruger et al., 1996; Ramacciotti et al., 2005), eating disorder comorbidity appears to not be limited to the behavioral features of aberrant eating

(i.e., binge eating, purging, dietary restriction) and may represent a marker for increased symptom load and illness burden (Wildes et al., 2007). Finally, epidemiological studies suggest an association between eating disorders and subthreshold bipolar symptoms, including affective temperaments, as well as between hypomania and binge eating behaviors, and the two disorders show considerable overlap in terms of phenomenology, course, comorbidity, family history, and pharmacologic treatment response (Lunde et al., 2009; McElroy et al., 2005).

This strong link between bipolar disorder and eating disorders may suggest a partially overlapping pathogenesis (McElroy et al., 2005), or it may imply that eating disorder comorbidity forms a specific subphenotype of bipolar disorder with a unique genetic architecture. Herein, we aimed to detect the genetic variants associated with increased risk for eating disorders in individuals with bipolar disorder through a GWA analysis.

METHODS

Subjects

Patients for this study were derived from the Bipolar Genome Study (BiGS). For genotyping as part of the BiGS, bipolar I subjects of European Ancestry were selected from those collected by the National Institute of Mental Health (NIMH) Genetics Initiative for Bipolar Disorder in five waves at 11 sites across the United States, as described elsewhere in detail (Dick et al., 2003; Smith et al., 2009). All subjects were assessed using the Diagnostic Interview for Genetic Studies (DIGS), which was combined with family informant data and medical records to arrive at best-estimate diagnoses according to DSM-III-R or DSM-IV criteria (Nurnberger et al., 1994). Control subjects were selected from those ascertained through an NIMH-supported contract mechanism between Dr. Pablo Gejman and Knowledge Networks, Inc. (Sanders et al., 2010). The selected controls were matched for gender and ethnicity (i.e., European Ancestry) with the bipolar patients, and all control subjects who endorsed a history of bipolar disorder, psychosis, or major depression on the medical questionnaire were excluded from this study.

Genotyping and Cleaning

The initial sample was genotyped at the Broad Institute as part of the as part of the Genetic Association Information Network (GAIN) using the Affymetrix 6.0 (1 M SNP) array. A total of 1,001 bipolar cases, 1,033 controls, and 724,067 SNPs were available for analysis following an extensive quality control (QC) process to eliminate subjects with ≥5% missing data and SNPs with ≥5% missing data, minor allele frequencies (MAF) <0.01, and Hardy-Weinberg Equilibrium $p<10^{-6}$ (Smith et al., 2009). A second sample of 1,198 bipolar cases and 403 controls was similarly genotyped at the Translational Genomics Institute (TGEN) and underwent a comparable QC process that resulted in 728,187 SNPs available for analysis (Smith et al., 2011). An additional round of QC performed on the merged GAIN and TGEN samples resulted in 703,012 passing SNPs. Identity by state (IBS) was used to identify and remove individuals with cryptic relatedness. The genetic homogeneity of the sample was assured by multidimensional scaling (MDS). All individuals were reported to be of European ancestry, and no population outliers were detected. The final bipolar cohort

(N=2190) is 58.7% female with an average age of 42.6±12.7, and the control cohort (N=1370) is 46.4% female with an average age of 52.2±17.1.

Phenotypes

Phenotypes for analysis were derived from the Phenome Database, which compiles data across the DIGS 2, 3, and 4 to arrive at a common set of variables for each subject and allow for diagnostic consistency among the different versions of the DIGS used for this sample (Potash et al., 2007). As part of the complete DIGS interview, all bipolar subjects were queried as to their eating habits and weight, and best estimate diagnoses of anorexia and bulimia nervosa were assigned. Bipolar patients were divided into the comorbid eating disorder (BD+ED) and bipolar disorder only (BDO) groups according to these diagnoses. Diagnostic crossover is very common within and among eating disorder diagnoses. Studies suggest that 20% to 50% of patients with a diagnosis of anorexia will develop bulimia over time (Eckert et al., 1995), 10% to 20% of those with bulimia will develop anorexia (Tozzi et al., 2005), and 62% of those diagnosed with the restricting-type of anorexia will switch to the binge-eating/purging-type (Eddy et al., 2002). The prevalence of these transitions suggests that anorexia and bulimia share genetic risk factors (Kendler et al., 1991; Schweiger and Fichter, 1997). Anorexia and bulimia are also cross transmitted in families, along with subthreshold forms of eating disorders, providing further evidence to suggest that these disorders are transmitted as a continuum of liability (Lilenfeld et al., 1998; Strober et al., 2000). We have thus combined anorexia and bulimia nervosa, as well as their subtypes, for an evaluation of the genetic determinants of eating disorder comorbidity within bipolar disorder.

The Childhood Life Event Scale (CLES) was administered at the time of interview for a portion of the cohort to document various traumatic events that may occur between the ages of 3–12 using a 12-point scale across 9 events. We rated this instrument as a cumulative total score and further evaluated subjects experiencing ≥2 events, per established methods (Anand et al., 2015). We also separately evaluated trauma in three categories: 1) death of a parent or sibling, having to leave home unexpectedly, and other serious life changing events; 2) beginning of a chronic illness, extended hospitalization (≥1 month), and permanent injury or disability; and 3) physical abuse and violent life threatening experiences. We note that some responses for the second category are confounded by the onset of mood symptoms in this cohort. The mean total CLES score across the 1,396 bipolar patients with valid data, including 131 patients with eating disorder comorbidity, was 1.4 ± 1.6 (range 0–10).

Statistical Analyses

An initial analysis comparing patients with comorbid eating disorders (BD+ED, N=184) to the healthy controls (CTL, N=1370) was performed to identify genetic factors that are unique to this subtype. In a complementary case-only analysis, the comorbid group was compared to the non-comorbid bipolar only group (BDO, N=2006) to identify genetic factors that may modify the expression of eating disorders within the context of bipolar disorder. These results were contrasted with those from an analysis comparing the non-comorbid group with controls to evaluate the specificity of the resultant associations for eating disorder comorbidity. Female sex-specific analyses for the 3q36.33 region included

158 BD+ED, 1127 BDO, and 636 CTL subjects. All association analyses were performed using logistic regression in PLINK (Purcell et al., 2007). As genomic inflation factors for the analyses ranged from 1.00 to 1.016, no correction for population stratification was deemed necessary. Label-switching permutations were performed to assess the empirical significance and stability of the results.

RESULTS

All bipolar patients were first evaluated for a variety of clinical characteristics related to binging and purging behaviors and patterns of dieting, exercise, and weight loss. Among the 2190 total bipolar patients, 272 (12.4%) had intentionally lost a lot of weight, and 320 (14.6%) had frequent eating binges as often as twice a week for at least three months. However, only 184 (8.4%) met full diagnostic criteria for an eating disorder, which included 66 (3%) with anorexia nervosa, 109 (5%) with bulimia nervosa, and 9 (0.4%) with eating disorder not otherwise specified. Among those with comorbid eating disorders, 15.8% were restricting type anorexia, 20.1% were binge eating/purging type anorexia, 39.7% were purging type bulimia, and 19.6% were non-purging type bulimia. As data suggests that anorexia and bulimia nervosa share genetic risk factors and exist on a continuum of liability (Kaye, 2008), we combined these diagnoses and their subtypes for an assessment of eating disorder comorbidity in bipolar disorder.

Given the evidence suggesting that eating disorder comorbidity may represent a marker for increased symptom load and illness severity, additional clinical characteristics of bipolar patients with and without eating disorder comorbidity were examined and are summarized in Table 1. Patients from the comorbid eating disorders (BD+ED) group had a significantly earlier age at onset of bipolar disorder than those from the non-comorbid bipolar only (BDO) group (14.4 vs. 19.3, $p<0.001$) and were much more likely to be female (85.9% vs. 56.2%, $p<0.001$). Rapid cycling was also present at a significantly elevated rate in the BD +ED group (71.7% vs. 55.0%, $p<0.001$). Furthermore, a significantly higher proportion of patients from the BD+ED group had experienced suicidal ideation (85.9% vs. 69.4%, $p<0.001$) and had attempted suicide (62.5% vs. 43.2%, respectively, $p<0.001$) than those without this comorbidity. While alcohol abuse was only moderately elevated in the BD+ED group (54.9% vs. 47.0%, $p=0.040$), significantly elevated comorbidity rates of panic disorder, agoraphobia, social phobia, and obsessive-compulsive disorder (OCD) were observed for the BD+ED group, as compared with the BDO group ($p\leq0.001$). Overall, anxiety spectrum disorders were found to co-occur with eating disorders in bipolar patients at a rate of 62.5% compared with a rate of 36.4% in the absence of eating disorders ($p<0.001$). Finally, patients of the BD+ED group appeared to have experienced more traumatic life events during their early childhood years, particularly events related to physical abuse or violence ($p<0.05$).

The results of the clinical comparisons suggest that eating disorder comorbidity may form a specific subphenotype of bipolar disorder. In order to differentiate genetic factors that are unique to the comorbid eating disorder subtype from those that modify the expression of eating disorders within bipolar disorder, the comorbid group (BD+ED) was compared to the healthy controls (CTL) and to the bipolar only group (BDO), respectively. These results

were then contrasted with the analysis of BDO vs. CTL to evaluate specificity for eating disorder comorbidity. The results of the genome-wide analyses are displayed in Figure 1. Genomic regions of interest were defined as those with at least two SNPs with $p<10^{-4}$ with additional support for association (i.e., $p<10^{-3}$) provided by surrounding SNPs within 100kb. A comprehensive list of regions meeting these criteria is provided in Supplementary Table S1 with a comparison of the statistics from all analyses.

The most significant genome-wide finding was observed for rs4854912 within the SOX2-OT region ($p=8.9\times10^{-8}$, odds ratio (OR)=1.9) on chromosome 3q26.33. While the overall best result was obtained for the BD+ED vs. CTL analysis, this region also produced the best finding in the BD+ED vs. BDO case-only analysis for a neighboring SNP (rs13086738, $p=2.1\times10^{-7}$, OR=1.8). Further support for this region derived from 13 SNPs in linkage disequilibrium that were associated in both analyses with p values $<10^{-5}$ (see Table S1). These SNPs spanned 235kb from SOX2-OT and extended into FXR1 (see Figure 2). Since the associated SNPs display an extremely high degree of linkage disequilibrium, we performed analyses conditional on rs4854912 and rs13086738 as appropriate. No other SNP in the 3q26.33 region remained significant, suggesting that this is a single association signal. Given the significantly larger proportion of females in the BD+ED group compared to the BDO and CTL groups, we repeated the analysis of this region both covarying for gender and in females only. The results remained similar, albeit slightly weaker, when gender was included as a covariate in the model for the BD+ED vs. CTL (rs4854912, $p=1.6\times10^{-6}$, OR=1.9) and BD+ED vs. BDO comparisons (rs13086738, $p=1.7\times10^{-6}$, OR=1.8). Still, the same 13 SNPs displayed evidence of association with p values$<10^{-4}$. The gender stratified analyses further confirmed that the size and direction of effect were consistent between females only and the complete sample for the BD+ED vs. CTL (rs4854912, $p=4.3\times10^{-5}$, OR=1.8) and BD+ED vs. BDO comparisons (rs13086738, $p=4.3\times10^{-5}$, OR=1.7), despite substantially reduced sample sizes.

Several other genomic regions of interest were also detected in these analyses (see Table S1 and Figure S1). Association to NALCN on chromosome 13q33.1 was observed most prominently for the BD+ED vs. CTL comparison (peak $p=6.0\times10^{-6}$, OR=1.7 for rs9554752), and the region of significance extended into the adjacent NALCN-AS1 gene, which encodes the antisense RNA of NALCN, with p values $<10^{-4}$. Other genes of interest that were most prominent in the BD+ED vs. CTL analysis included NRF1 on 7q32.2, NRG3 on 10q23.1, and ADNP on 20q13.13. The ABCG1 gene on chromosome 21q22.3 was most prominent in the BD+ED vs. BDO analysis (peak $p=4.3\times10^{-7}$). Genes of interest in the analyses of BD+ED vs. BDO and vs. CTL included CADM3 on chromosome 1q23.2, ATP2B4 on 1q32.1, and RYR2 on 1q43.

DISCUSSION

Bipolar disorder shows significant clinical phenotypic heterogeneity, which may reflect differences in the underlying genetic architecture. The use of clinical features to refine the diagnosis and reduce the phenotypic heterogeneity may provide additional power to detect genetic risk variants (Manchia et al., 2013). Based on prior evidence suggesting a partially overlapping pathogenesis, we investigated bipolar disorder with eating disorder comorbidity

through GWA analyses towards the identification of variants contributing either uniquely to this subphenotype or to risk for both bipolar and eating disorders (McElroy et al., 2005).

The lifetime prevalence rates of 3% for anorexia nervosa and 5% for bulimia nervosa observed in our sample are consistent with prior estimates of these eating disorders in bipolar disorder and are significantly elevated in comparison with the rates of 0.3–0.7% and 1.5–2.5% observed for these disorders, respectively, in the general population (Kaye, 2008; McElroy et al., 2011). Consistent with the findings of others, presence of a lifetime comorbid eating disorder in our study was associated with female gender, an earlier age at onset of bipolar disorder, and a history of rapid cycling and suicide, all of which predict a more severe course of illness (Brietzke et al., 2011; McElroy et al., 2011). While anxiety disorders often show comorbidity with bipolar disorder (McElroy et al., 2001), we find a dramatic increase in the comorbidity of panic disorder, OCD, and social phobia in bipolar patients with eating disorder comorbidity, consistent with prior observations (Jen et al., 2013). However, this may not be unexpected given the relationship between anxiety and eating disorders aside from the context of bipolar disorder (Kaye, 2008). Finally, childhood trauma has been implicated as a precursor to both bipolar disorder and eating disorders, with a particular emphasis on physical abuse as a trigger (Jen et al., 2013; Leverich et al., 2002; Post and Leverich, 2006; Rayworth et al., 2004; Rodriguez et al., 2005). Our data provides further evidence to support this connection, with an increased incidence of traumatic events in childhood for bipolar patients with eating disorder comorbidity, an effect that seems to be driven primarily by experiences of physical abuse and violence. Further evaluation of the interaction of trauma exposure as an early environmental risk factor and particular genetic risk factors will be necessary to understand the relationship between trauma, bipolar disorder, and eating disorder comorbidity.

The most significant genome-wide finding was observed for the SOX2-OT/FXR1 region on chromosome 3q26.33 in the BD+ED vs. CTL analysis with a peak p value of 8.9×10^{-8}. This region also featured prominently the BD+ED vs. BDO comparison with a peak p value of 2.1×10^{-7}. For both analyses, a total of 13 SNPs in high linkage disequilibrium spanning 235kb provided support for association with p values $<10^{-5}$. The chromosome 3q26-27 region has previously been implicated in linkage studies of bipolar disorder (Badenhop et al., 2002; Kelsoe et al., 2001). A recent large GWA study of 2,907 cases and 14,860 controls also reported a significant association between SOX2-OT and anorexia nervosa (peak $p=3.0\times10^{-7}$, OR=1.2 for rs9839776) (Boraska et al., 2014). Although located within the same large gene, rs9839776 is approximately 500kb from the associated region in our analyses. Still, given the exclusive association of SOX2-OT with the BD+ED group in our study, these combined results may either suggest a role for this gene in eating disorders or provide evidence for a commonality of genes underlying bipolar disorder and eating disorders. Interestingly, a recent very large study of schizophrenia produced two genome-wide significant hits to the 3q26.33 region, one for an insertion/deletion polymorphism in FXR1 (OR=0.91, $p=1.3\times10^{-11}$) and another for a SNP in SOX2-OT (rs9841616, OR=0.92, $p=1.65\times10^{-8}$), with many SNPs providing additional support for association (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). This region thus warrants further investigation as harboring one or more genes common to the etiology of several psychiatric disorders.

SOX2-OT encodes for the SRY-box containing gene 2 (SOX2) overlapping transcript and was identified as a spliced, long non-protein-coding RNA (lncRNA) with an intron overlapping the SOX2 gene in the same transcriptional orientation. SOX2-OT may regulate the expression of SOX2, the product of which plays a key role in both embryonic and adult neurogenesis (Amaral et al., 2009). SOX2-OT also appears to represent a biomarker for the early and late stages of neurodegeneration (Arisi et al., 2011), and an isoform of SOX2-OT transcribed from a distal, highly conserved element located within the region of our association peak is exclusively expressed in the brain with enrichment in regions of adult neurogenesis (Amaral et al., 2009). FXR1 is an autosomal homolog of Fragile X mental retardation-related protein 1 (FMR1), which is responsible for the Fragile-X syndrome in humans (Ashley et al., 1993; Siomi et al., 1993; Siomi et al., 1995). During embryonic development, the expression of FXR1 is restricted to early stages of proliferation and differentiation in regions of the central nervous system, suggesting that FXR1 may play an important role in nerve cell proliferation and early brain development (Coy et al., 1995).

Several other genomic regions with p values ranging from 10^{-5} to 10^{-7} were identified in the BD+ED vs. CTL analysis, which may suggest variants that are unique to a comorbid eating disorder subtype of BD. These included NALCN and its antisense RNA on chromosome 13q32-33. NALCN encodes a nonselective sodium leak channel that plays an important role in the regulation of neuronal excitability (Cahoy et al., 2008; Lu et al., 2007). Suggestive evidence for linkage to bipolar disorder has been observed for chromosome 13q31-34 (Kelsoe et al., 2001; Potash et al., 2003; Shaw et al., 2003), and GWA further implicates NALCN in bipolar disorder (Ollila et al., 2009; Wang et al., 2010). Several of the identified genes are involved in the regulation of neurodevelopment and neuroplasticity. NRF1 on chromosome 7q32.2 encodes nuclear respiratory factor 1 and has been shown to regulate neurite outgrowth (Wang et al., 2009; Wang et al., 2013). NRG3 (neuregulin 3) on chromosome 10q23.1 plays a critical role in the development of the embryonic cerebral cortex (Meier et al., 2013), and variants of this gene are associated with cognitive deficits in bipolar disorder and schizophrenia (Meier et al., 2013). ADNP on chromosome 20q13.13 encodes an activity-dependent neuroprotective protein that appears essential for neuronal differentiation, neurodevelopment, and neuroprotection, with abundant expression in hippocampus, hypothalamus, cerebral cortex, and cerebellum (Aboonq et al., 2012; Oz et al., 2014; Pinhasov et al., 2003; Yang et al., 2012). Finally, several genes spanning chromosome 1q23-43 are involved in the regulation of calcium homeostasis and neurodevelopment, including CADM3 (cell adhesion molecule 3 isoform 1), ATP2B4 (plasma membrane calcium ATPase isoform 4), and RYR2 (ryanodine receptor 2) (Galeotti et al., 2008; Kakunaga et al., 2005; Tempel and Shilling, 2007; Zalk et al., 2007). Calcium is a ubiquitous signaling molecule that plays a crucial role in the regulation of neurotransmitter release, synaptic plasticity, neurite outgrowth, and neurodegeneration (Berridge, 1998; Ciccolini et al., 2003), and several studies have suggested an altered calcium homeostasis in the pathophysiology of bipolar disorder (Emamghoreishi et al., 2000; Yoon et al., 2001).

The case only analysis, which was intended to identify genes modifying the expression of eating disorder comorbidity in bipolar disorder, implicated ABCG1 on chromosome 21q22. This region has previously been implicated in genetic linkage studies of bipolar disorder (Aita et al., 1999; Kaneva et al., 2004; Straub et al., 1994), and ABCG1 has been suggested

as both a positional and functional candidate gene for bipolar disorder (Kirov et al., 2001).. ABCG1 encodes the ATP-binding cassette sub-family G member 1, a transporter protein involved in the cellular uptake of tryptophan, the precursor for serotonin, which is involved in the pathophysiology of mood disorders. Serotonin is also a key regulator of eating behavior, and genetic variants contributing to serotonergic dysfunction impact risk for eating disorders (Kaye, 2008; Lucki, 1998).

The regions of interest in the present study did not include genes identified by previous GWA studies of bipolar disorder, such as ADCY2, ANK3, CACNA1C, NCAN, ODZ4, and TRANK1 (Cichon et al., 2011; Ferreira et al., 2008; Green et al., 2013; Chen et al., 2013; Muhleisen et al., 2014; Psychiatric GWAS Consortium Bipolar Disorder Working Group, 2011). These regions also did not include genes that have been reported to be associated with anorexia nervosa, such as CTNNA2, CNTNAP2, EPHX2, GABRG1, HTR1D, OPRD1, and PPP3CA (Bloss et al., 2011; Boraska et al., 2014; Scott-Van Zeeland et al., 2014; Wang et al., 2011). This, in combination with the identification of genes in the BD+ED analyses that were not significant in the BDO vs. CTL analysis, suggests that bipolar disorder with eating disorder comorbidity may represent a unique clinical phenotype that is distinct from both bipolar disorder and eating disorders. Our analyses also appear to broadly support the involvement genes related to neurodevelopment and neuroprotective mechanisms in pathophysiology of bipolar disorder and the modification of the expression of this clinical subtype (Harwood, 2003; Rowe and Chuang, 2004; Sanches et al., 2008; Soeiro-de-Souza et al., 2012).

There are limitations to the present study related primarily to sample size. The BD+ED group was relatively small with 184 subjects, although the comparison groups were substantially larger. Still, we may lack sufficient power to detect smaller genetic effects with confidence. The estimation of p values may also be distorted in smaller samples, although the permutation analyses confirmed the stability of our results and provided comparable estimates of association. Additionally, the use of all available subjects from the GAIN and TGEN cohorts combined to increase the sample size of the BD+ED group precluded a replication effort. However, we view our study as a replication and confirmation of the recent findings of Boraska and colleagues (Boraska et al., 2014), which implicated SOX2-OT in a GWA of anorexia nervosa with a similar p value (3.01×10^{-7}) in a much larger cohort. Given that our p values were comparable to those of Boraska and that the direction of effect was the same for our BD+ED analyses as for anorexia vs. controls, we feel our study substantially strengthens the confidence of the involvement of SOX2-OT in the etiology of eating disorders. Finally, it must be noted that a prior study of the GAIN portion of this sample by Winham and colleagues reported an association for the APOB gene with binge eating behavior in bipolar disorder that we were unable to confirm (Winham et al., 2014). This discrepancy is likely due to several factors. First, we have added the TGEN cohort, nearly doubling the overall sample size, although possibly introducing genetic heterogeneity due to differences in ascertainment methods between the bipolar cohorts (Greenwood and Kelsoe, 2013). Second, we relied on a more stringent definition of eating disorders, requiring a confirmed diagnosis for categorization as comorbidity, whereas the Winham study categorized subjects according to a broader binge eating phenotype via a single DIGS question (Winham et al., 2014). As an example, our study identified only 60

patients with BD+ED from the GAIN cohort with the remaining 124 derived from TGEN, whereas the Winham study identified 206 bipolar patients with binge eating behavior in the GAIN cohort alone. Lastly, we used a combined eating disorder diagnosis that included both anorexia and bulimia to increase the sample size, which may have introduced genetic heterogeneity and divergence from the prior study. We thus expect different results from these studies employing alternative phenotype definitions in overlapping yet distinct cohorts.

In summary, we confirmed the association of a recently identified candidate gene for anorexia nervosa in bipolar patients with eating disorder comorbidity. We also identified several genomic regions of interest containing genes involved in neurodevelopment and neuroprotection processes that may be relevant to the specific pathophysiology of eating disorder comorbidity in bipolar disorder or may represent part of the shared pathophysiology underlying both bipolar disorder and eating disorders. Although these findings require confirmation in larger datasets, they support the concept that different clinical manifestations of bipolar disorder may reflect differences in the underlying genetic architecture.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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HIGHLIGHTS

- Genome-wide association of eating disorders within bipolar disorder was evaluated.
- These results replicate the prior association of SOX2-OT with eating disorders.
- Genes involved in neurodevelopment and neuroprotection were also identified.
- Clinical subtypes of bipolar disorder may reflect distinct genetic contributions.

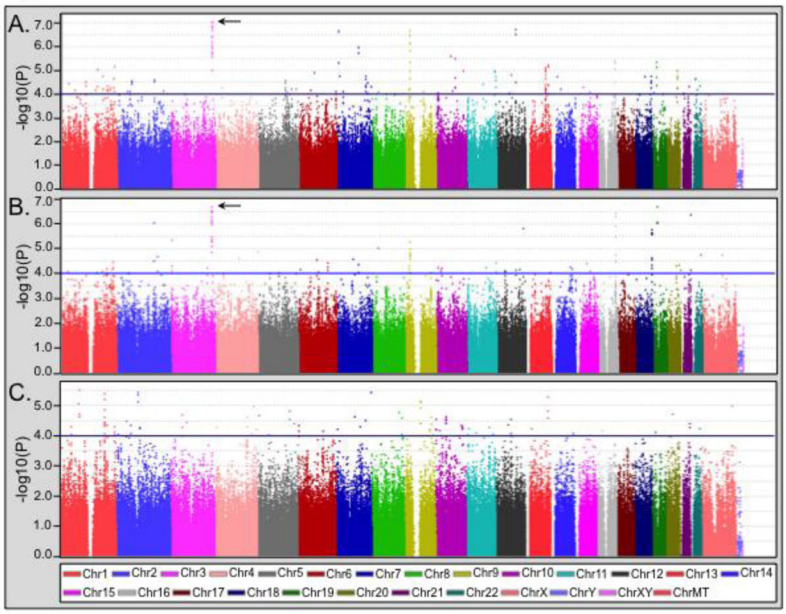

Figure 1.

Results of the genome-wide association analysis of bipolar with comorbid eating disorder (BD+ED) vs. healthy controls (CTL) and the noncomorbid bipolar only group (BDO). (A) Analysis of BD+ED (N=184) vs. CTL (N=1370). (B) Case-only analysis of BD+ED vs. BDO (N=2006). (C) The analysis of BDO vs. CTL is provided for comparison. In each panel, a blue line indicates $p<10^{-4}$, and an arrow indicates the 3q26.33 region.

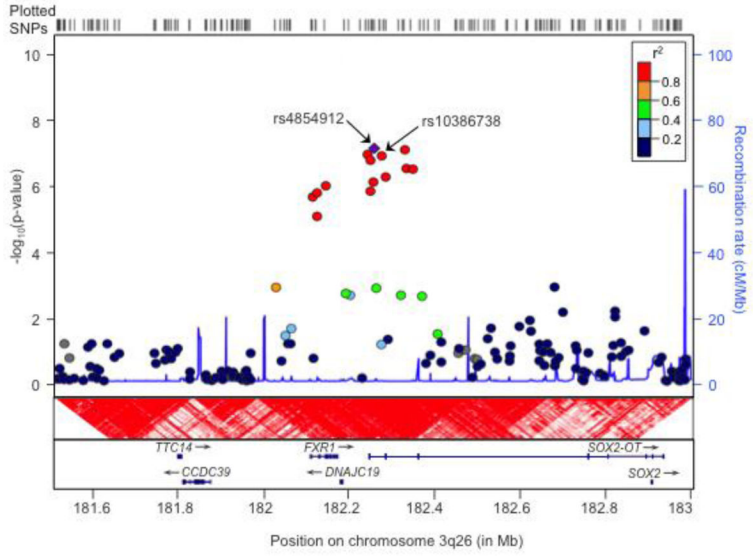

Figure 2.

Details of the 3q26 region from LocusZoom (Pruim et al., 2010), displaying the chromosomal context, linkage disequilibrium structure, and patterns of recombination surrounding the peak SNP, rs4854912, which is shown as a purple diamond. All locations are based on the hg 18 assembly, and linkage disequilibrium patterns across the region are shown according to the CEPH reference population from the HapMap release 22 with red indicating complete disequilibrium ($D'=1$). The association results shown correspond to the BD+ED vs. CTL analysis (see Figure 1A), with a peak p value of 8.9×10^{-8} observed for rs4854912. Nearby genes SOX2-OT and FXR1 are also shown. The BD+ED vs. BDO analysis produced a very similar regional association plot, and the peak SNP from that analysis, rs10386738, is also indicated.

Table 1

Clinical characteristics of bipolar patients with and without comorbid eating disorders.

	BD comorbid ED (N=184)	BD only (N=2006)	Statistics
Female Gender	158 (85.9%)	1127 (56.2%)	$\chi^2(1)=61.3$, $p<0.001$
Age at Onset of Bipolar Disorder	14.4 ± 6.6	19.3 ± 9.5	$t=9.0$, $p<0.001$
Global Assessment of Functioning	61.7 ± 14.6	64.0 ± 16.0	$t=1.8$, $p=0.072$
Panic Disorder	75 (40.8%)	481 (24.0%)	$\chi^2(1)=25.1$, $p<0.001$
Agoraphobia	47 (25.5%)	280 (14.0%)	$\chi^2(1)=17.8$, $p<0.001$
Social Phobia	38 (20.7%)	240 (11.9%)	$\chi^2(1)=11.5$, $p=0.001$
Obsessive-Compulsive Disorder (OCD)	50 (27.2%)	190 (9.5%)	$\chi^2(1)=54.1$, $p<0.001$
Alcohol Abuse	101 (54.9%)	943 (47.0%)	$\chi^2(1)=4.2$, $p=0.040$
Substance Abuse	29 (15.8%)	328 (16.4%)	$\chi^2(1)=0.04$, $p=0.836$
Rapid Cycling	132 (71.7%)	1104 (55.0%)	$\chi^2(1)=15.9$, $p<0.001$
Suicidal Ideation	158 (85.9%)	1393 (69.4%)	$\chi^2(1)=16.8$, $p<0.001$
Suicide Attempt	115 (62.5%)	871 (43.2%)	$\chi^2(1)=24.7$, $p<0.001$
Number of Traumatic Events in Childhood [a]	1.7 ± 1.6	1.4 ± 1.6	$t=-2.1$, $p=0.040$
Experience of ≥2 Traumatic Events in Childhood [b]	62 (47.3%)	473 (37.4%)	$\chi^2(1)=5.1$, $p=0.023$
Experience of a Life Changing Event in Childhood [c]	61 (46.6%)	472 (37.3%)	$\chi^2(1)=4.3$, $p=0.038$
Experience of a Chronic Illness, Hospitalization, or Injury in Childhood [d]	25 (19.1%)	217 (17.2%)	$\chi^2(1)=0.3$, $p=0.579$
Experience of Physical Abuse or Violence in Childhood [e]	34 (26.0%)	222 (17.5%)	$\chi^2(1)=5.6$, $p=0.018$

[a] Total number of traumatic events experienced between the ages of 3 and 12, as defined by the Childhood Life Events Scale (CLES). Note that 131 subjects with BD+ED and 1265 subjects with BD only have CLES data.

[b] Experience of 2 or more traumatic events across the entire CLES.

[c] Experience of trauma related to the death of a parent or sibling, leaving home unexpectedly, and other serious life changing events.

[d] Experience of trauma related to the beginning of a chronic illness, extended hospitalization (≥1 month), or permanent injury or disability. Note that this category is confounded by the report of onset of mood symptoms for many patients.

[e] Experience of trauma related to physical abuse or violent life threatening experiences.